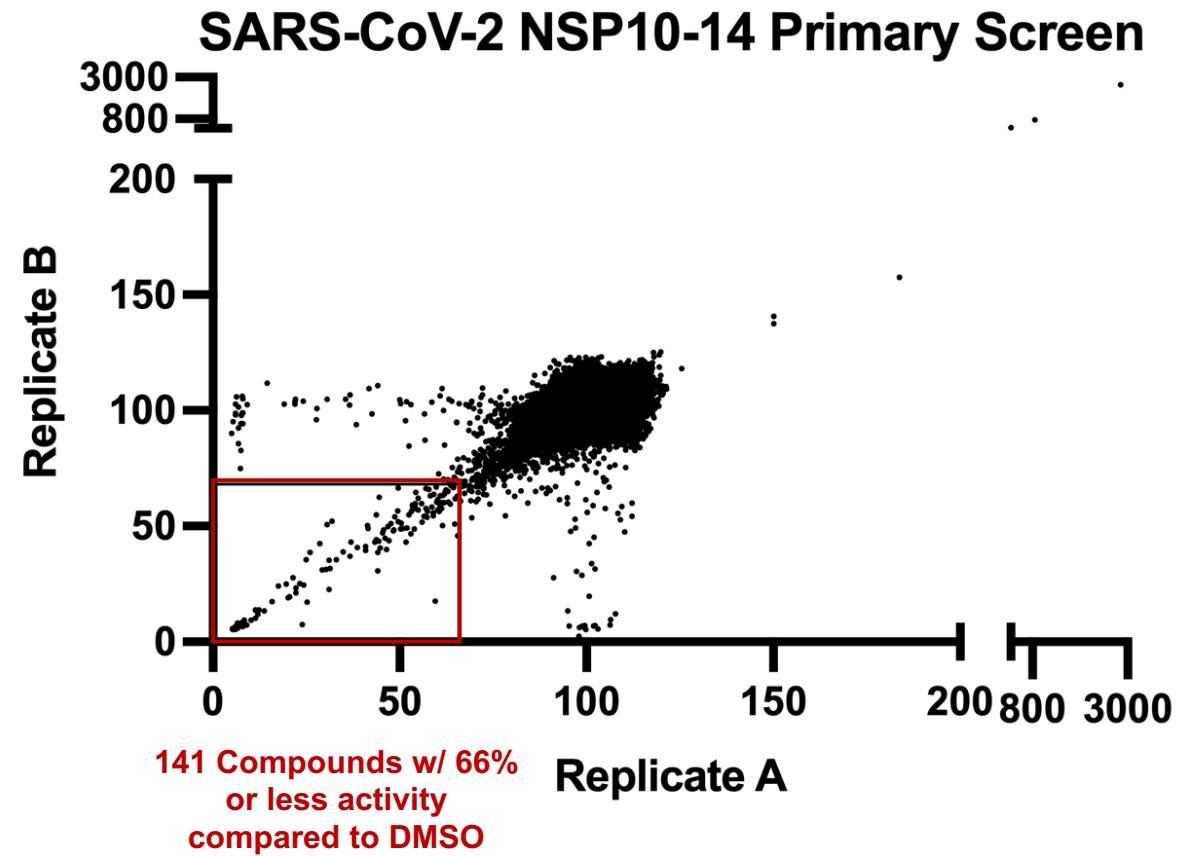
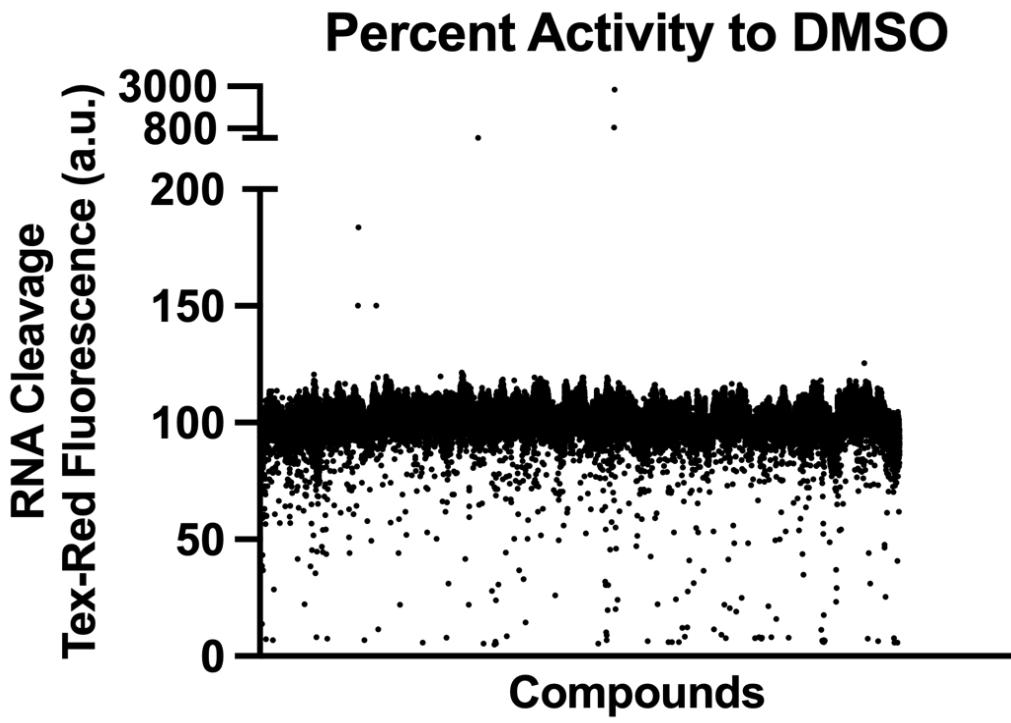


Identification of Novel Antiviral Compounds against Coronavirus Exonuclease NSP14



Mackenzie L. Anderson, Ph.D.
Heaton Laboratory
Duke University School of Medicine

Identification of NSP14 Inhibitors with TimTec AG50K Library



Average Screen Z-Factor = 0.93

Compound Validation and 4-Point IC₅₀ Curve Generation – Additional Compounds

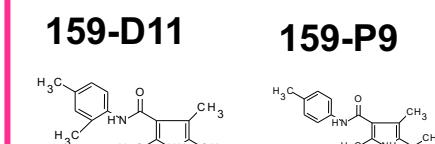
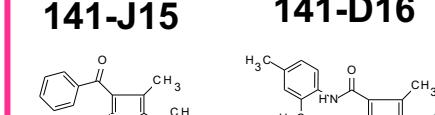
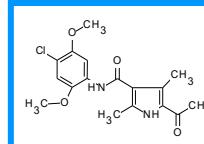
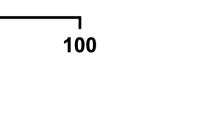
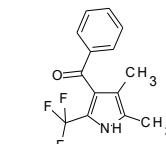
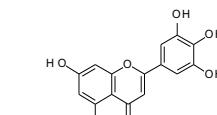
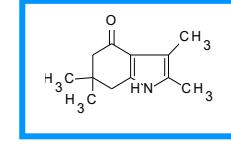
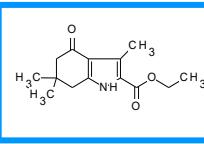
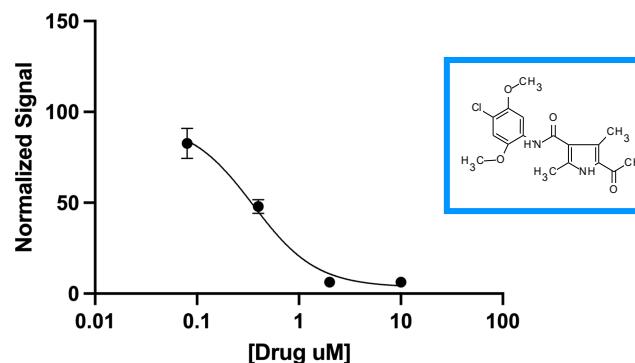
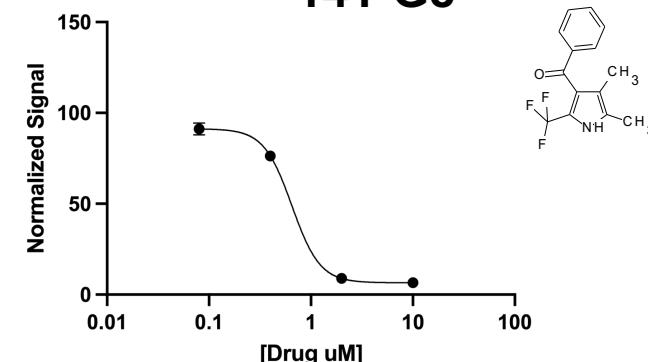
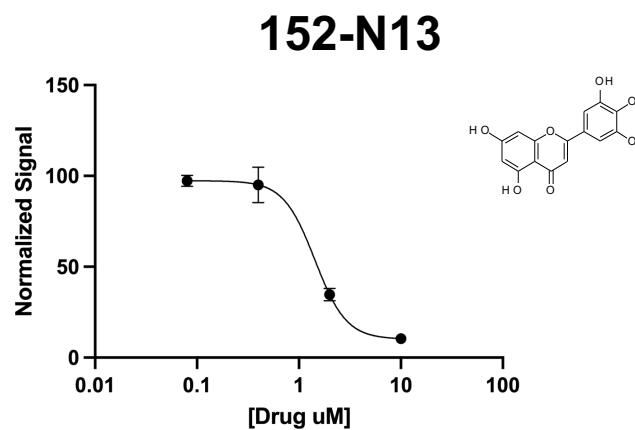
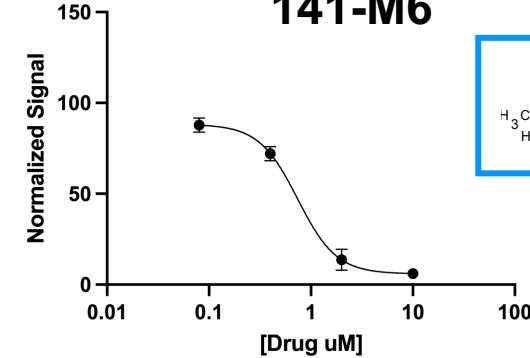
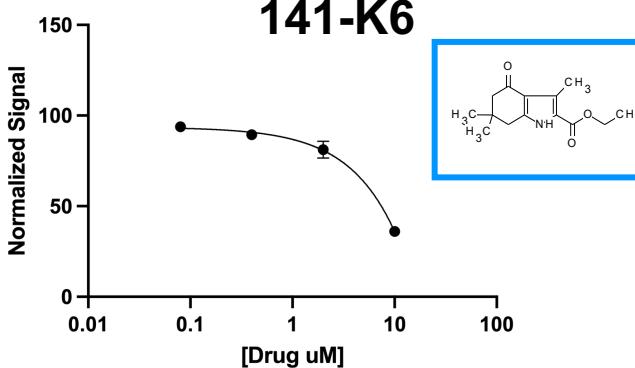
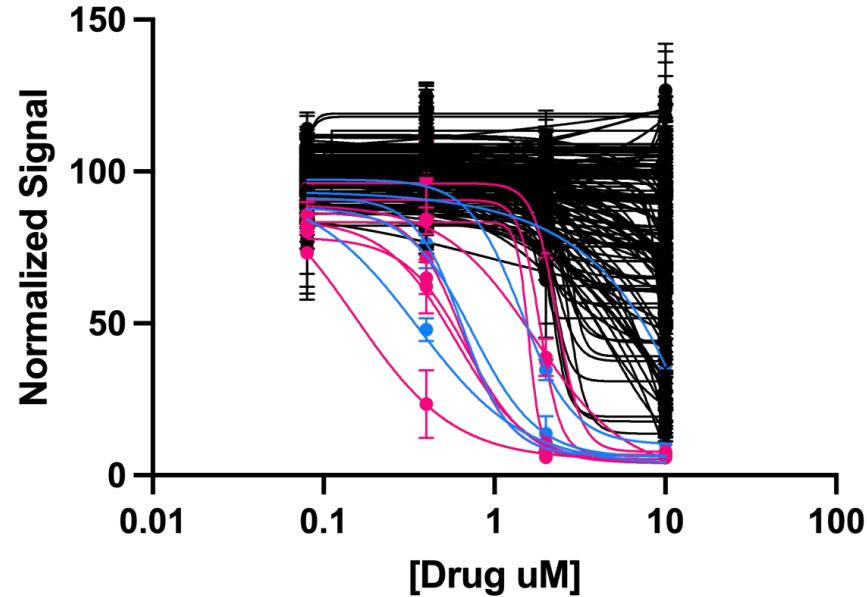


Reordered
compounds - custom
96-well plates w/ top
141 hits
(<66% activity)

Measured % RNA Cleavage normalized to DMSO

10 μ M 2 μ M 0.4 μ M 0.08 μ M

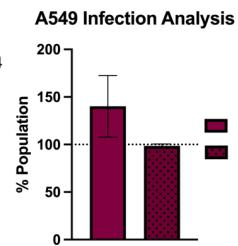
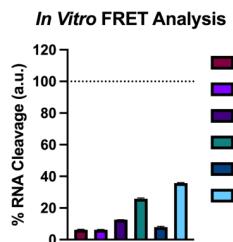
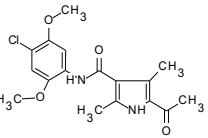
4 Point IC₅₀ Analysis



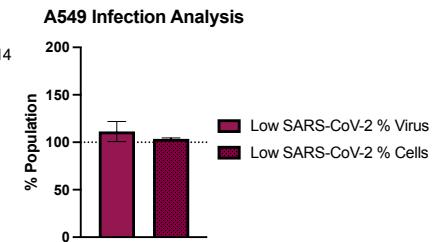
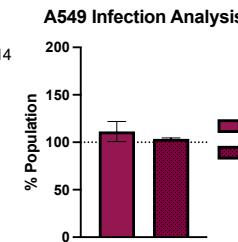
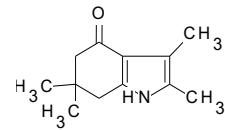
Top
compounds
from last
time (pink
curves)

Characterization of Additional TimTec Compounds

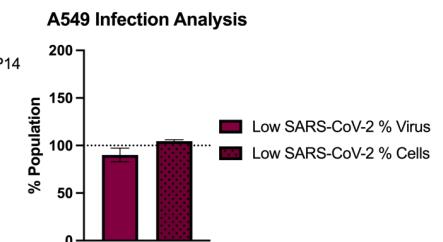
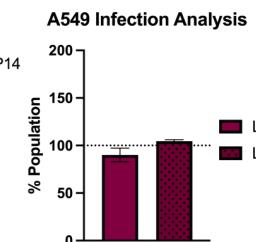
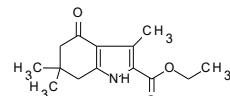
141-H16



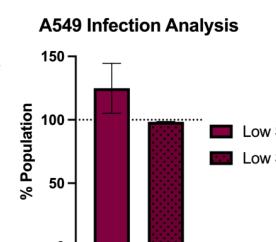
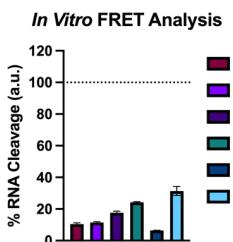
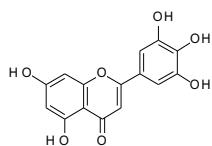
141-M6



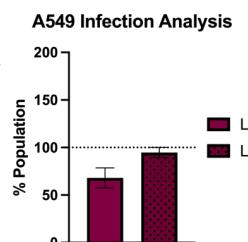
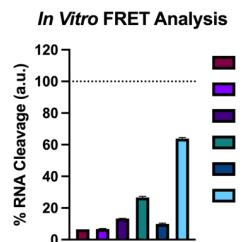
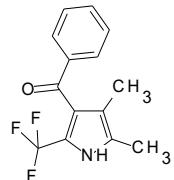
141-K6



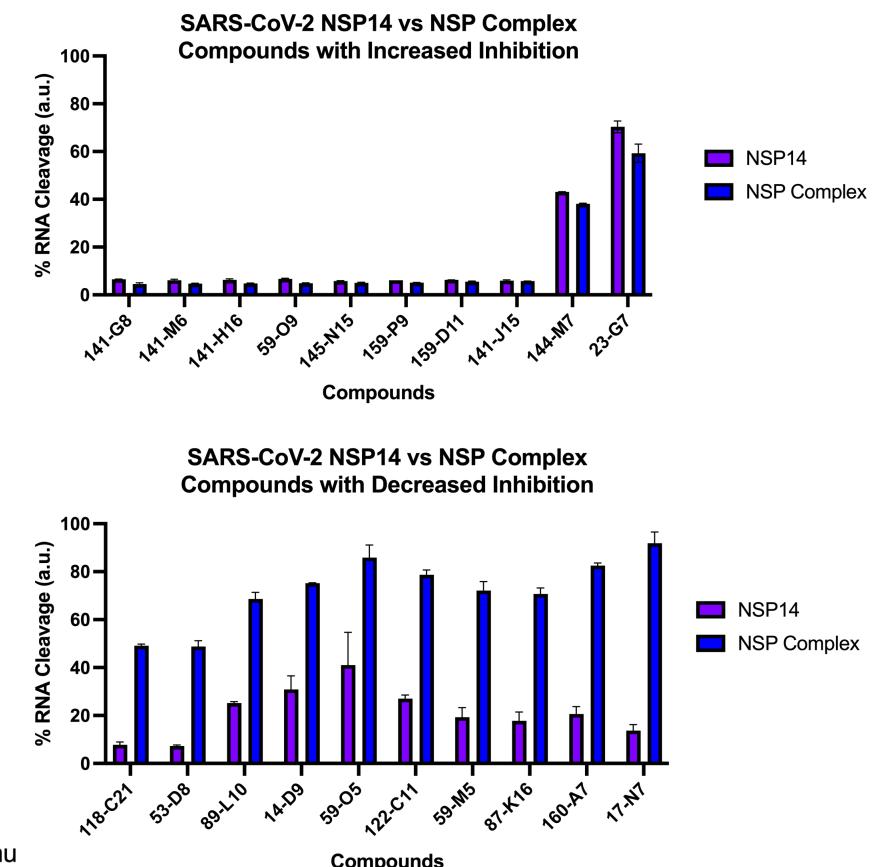
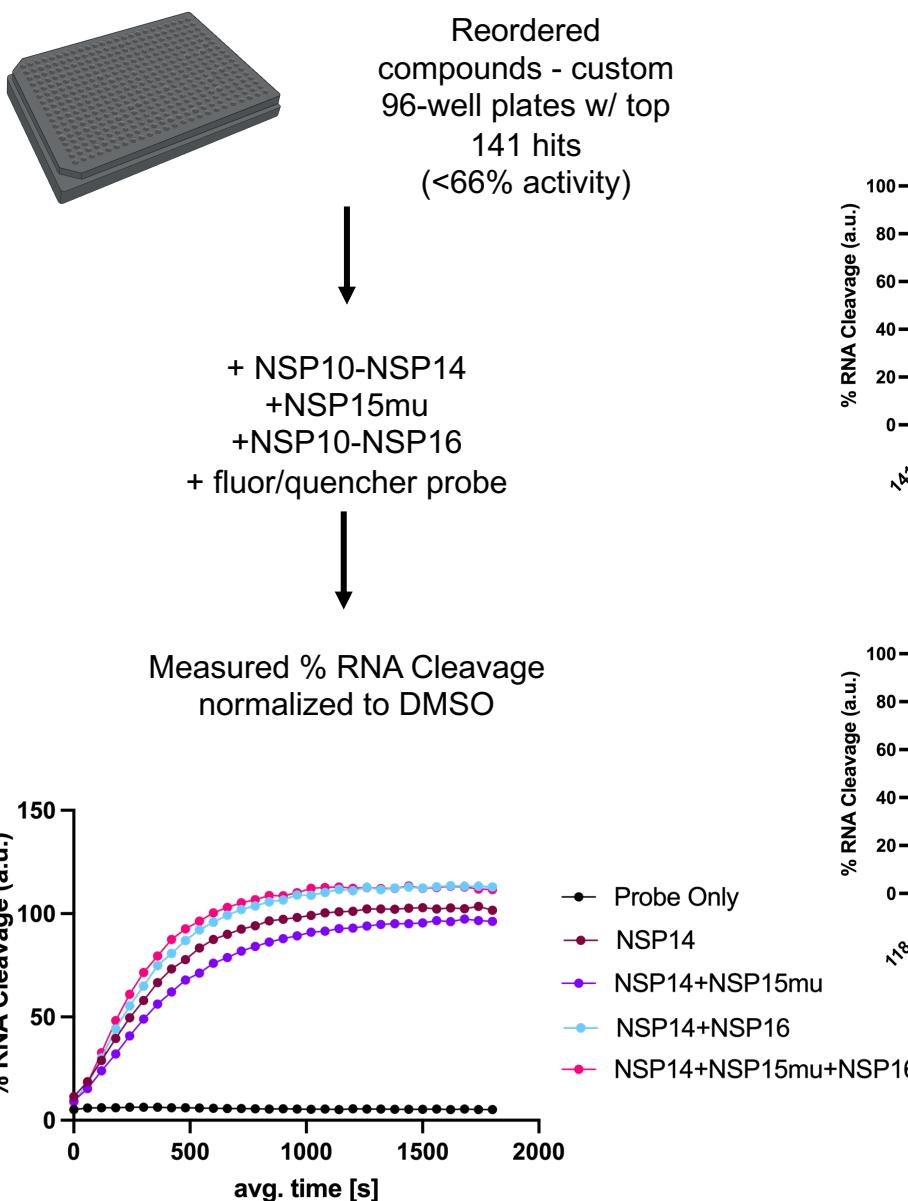
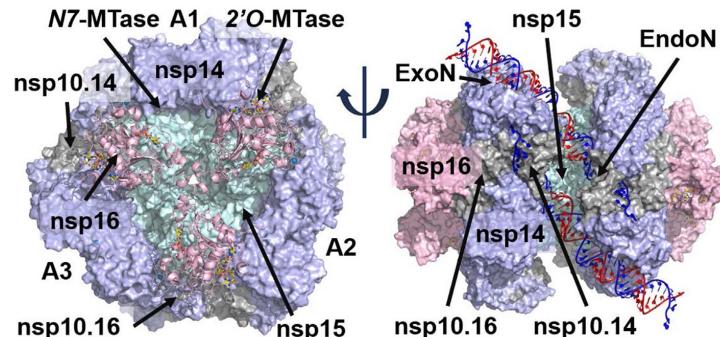
152-N13



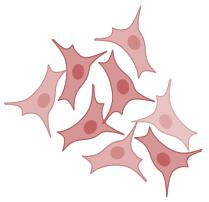
141-G8



Analysis of Compound Activity in Presence of NSP Complex



Remdesivir IC₅₀ Curves w/ Top FRET Compounds and OC43



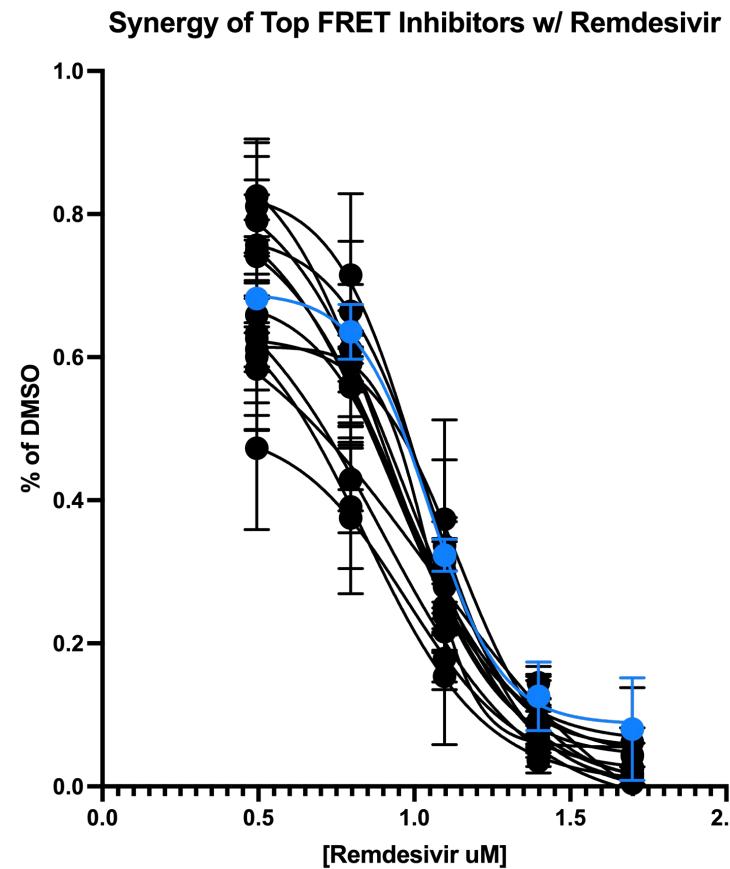
Day 1:
Plate A549 cells in 96 well plates



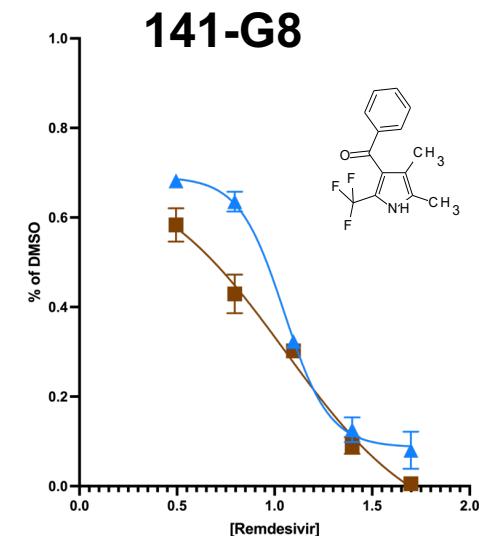
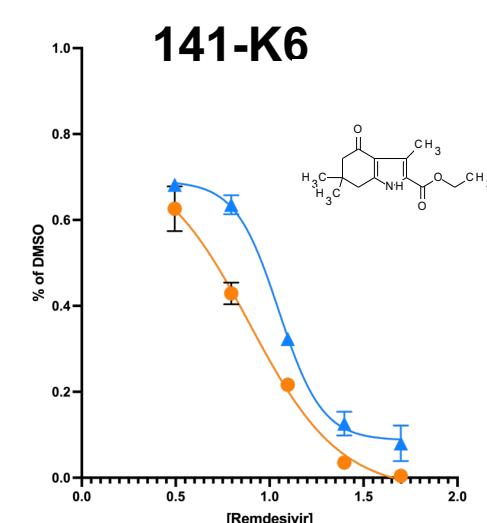
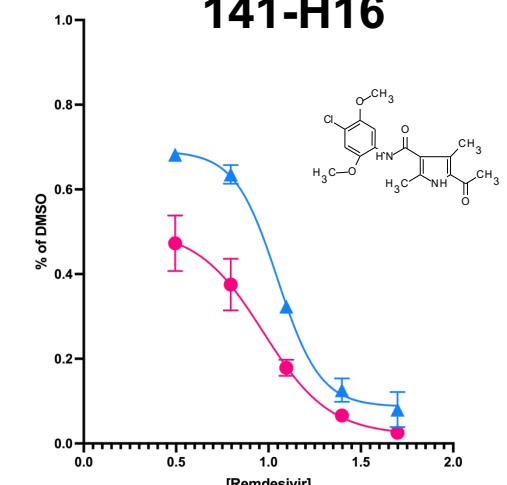
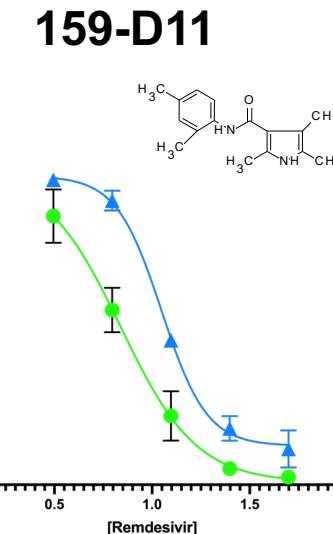
Day 2:
Pre-treatment of A549 cells with 30 uM Compound + varying concentrations of Remdesivir

OC43
Addition of 50 μ L DMEM w/ 2% FBS to pretreatment;
Incubation at 37 °C for 30 hr.

Day 3:
Fix and stain for microscopy analysis
OC43: N protein

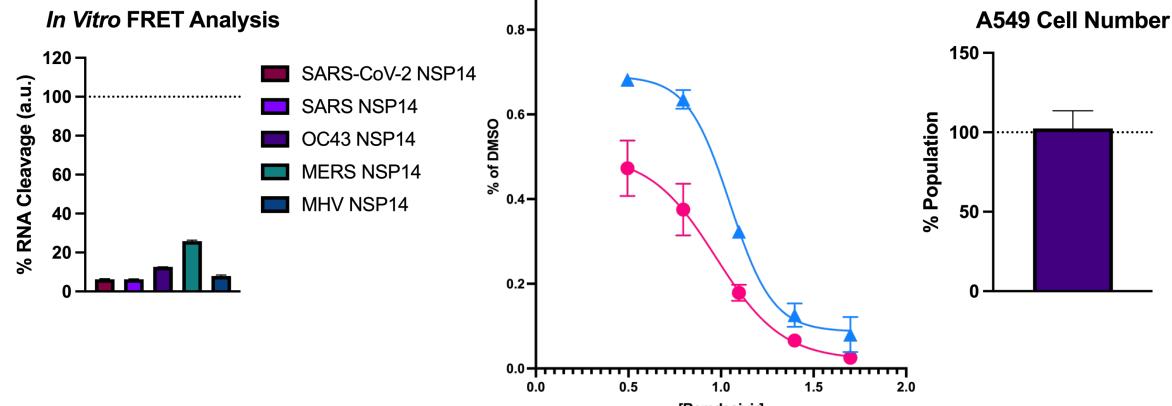


*Concentration is log transformed

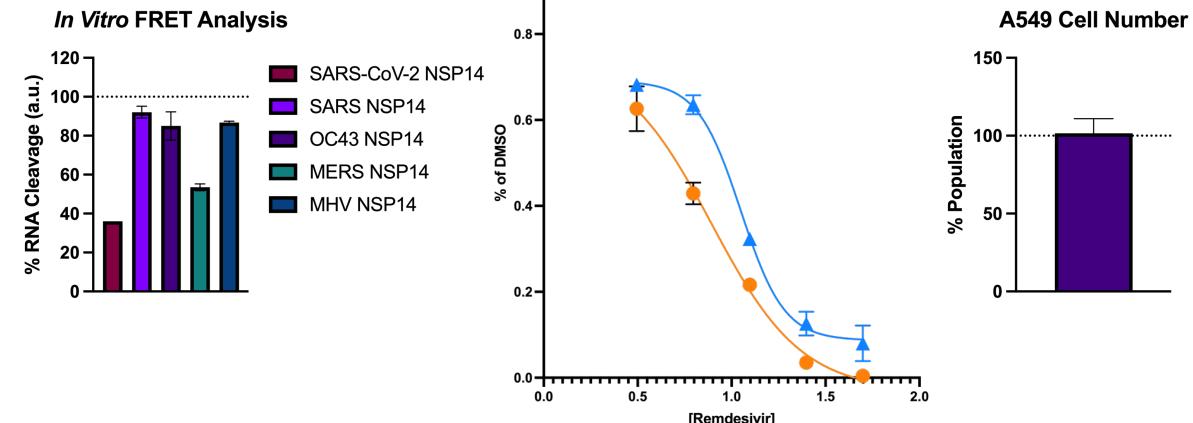


Compounds that Shift Remdesivir IC₅₀

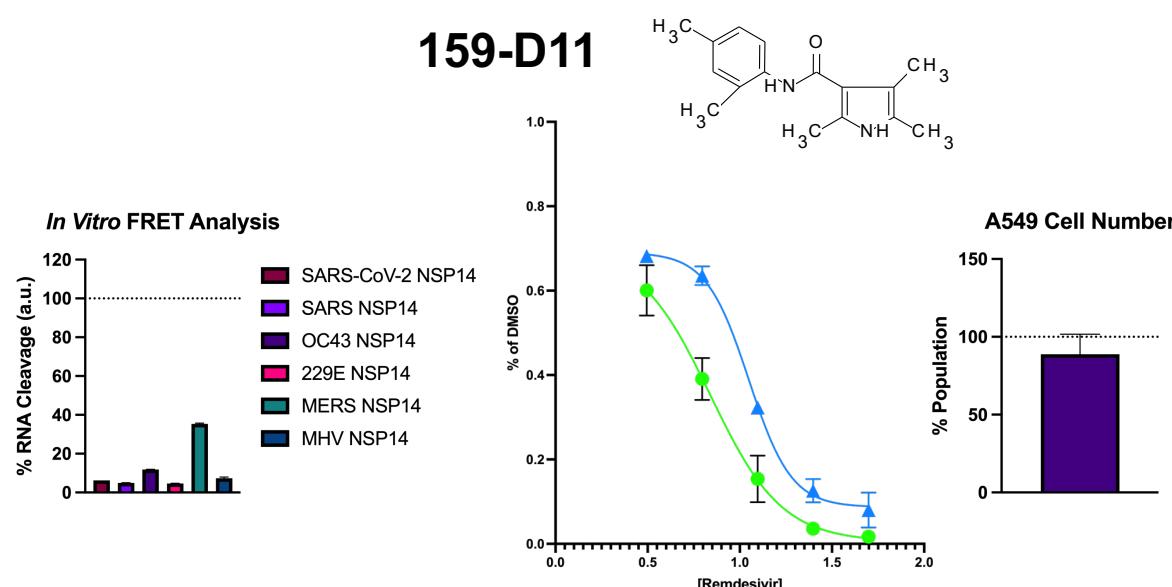
141-H16



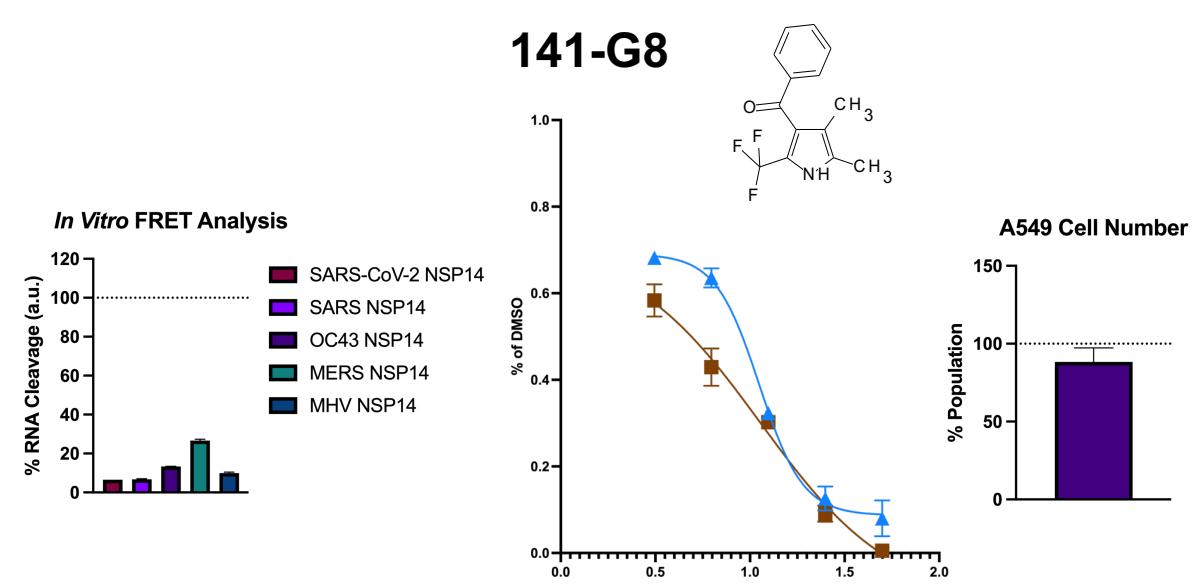
141-K6



159-D11



141-G8



Ongoing & Future Experiments:

Cellular Validation

- IC₅₀ generation of top 7 antiviral compounds showing direct activity against SARS-CoV-2
- Synergy of top 14 FRET compounds with Remdesivir in A549 ACE cells (GS-441524) w/ SARS-CoV-2

Further IC₅₀ Validation

- 12-Point IC₅₀ curves *in vitro* with top FRET compounds for more precise values

Future Experiments:

- Specific infectivity experiments after serial growth in compounds
- Potentiation of chain-terminator nucleoside analogues